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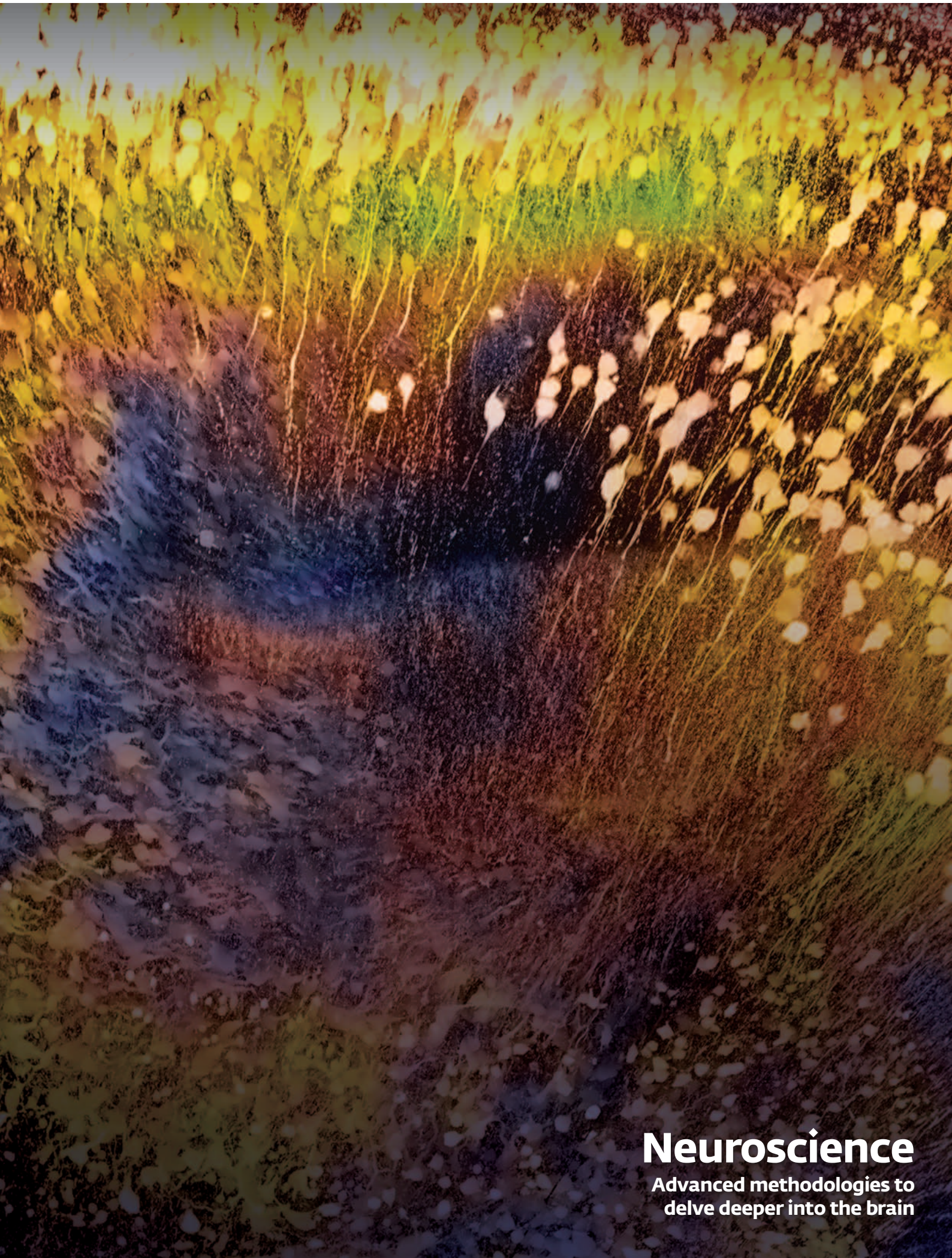
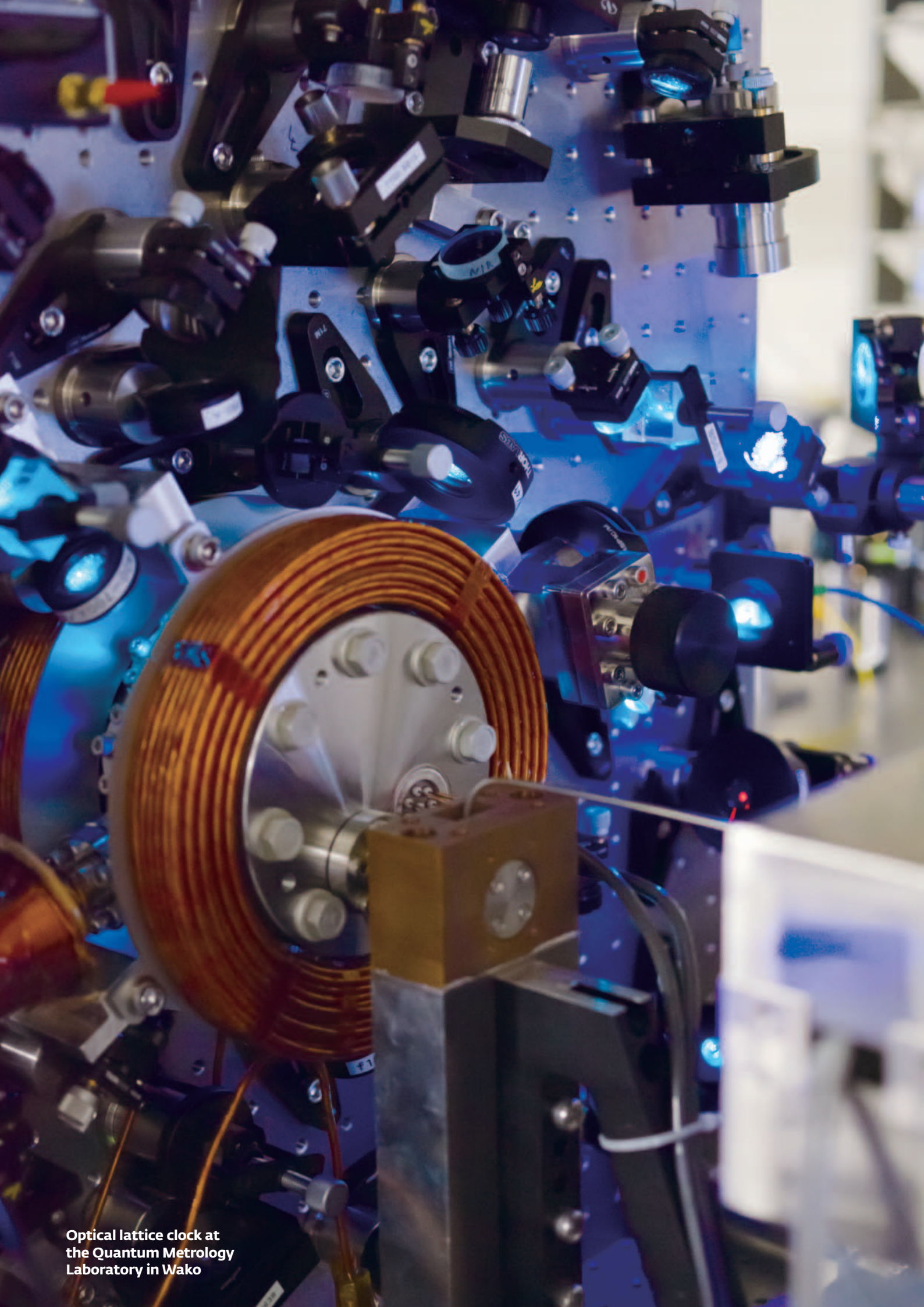


Neuroscience

Advanced methodologies to delve deeper into the brain



Optical lattice clock at the Quantum Metrology Laboratory in Wako

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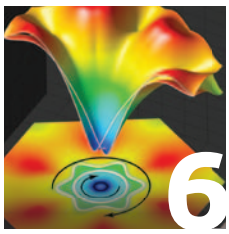

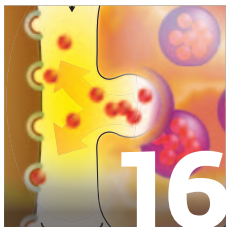

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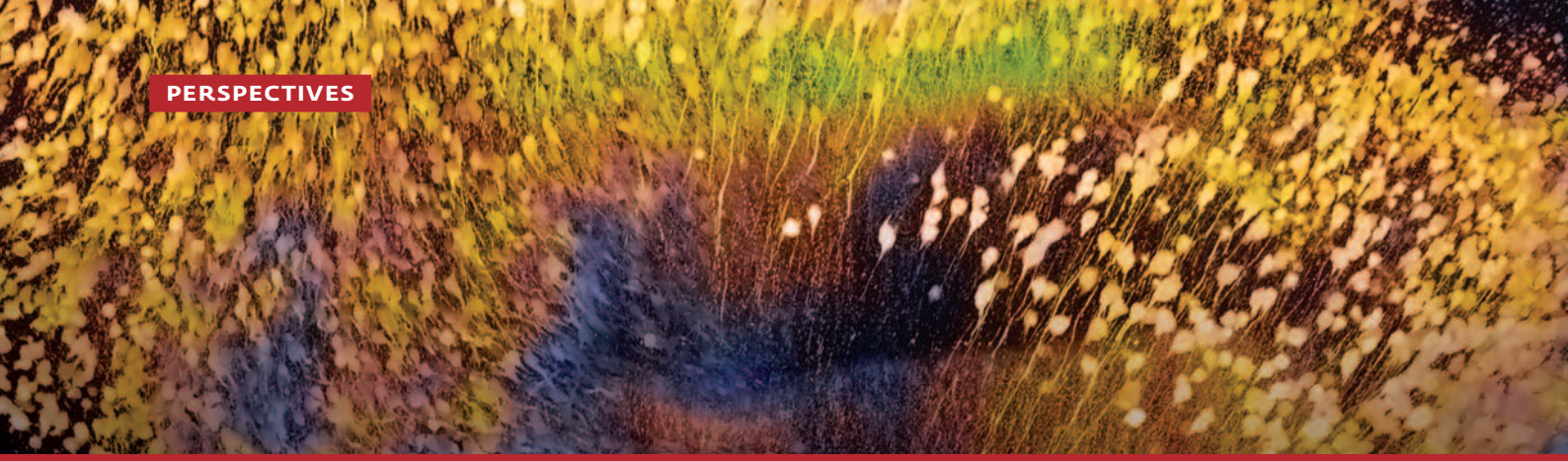

Neuroscience

Revealing the secrets of the brain

SUSUMU TONEGAWA
Director
RIKEN Brain Science Institute

The human brain is our most complex organ. Decades of research are starting to reveal how brain cells work and interact to form networks, how these networks generate thoughts and behavior and how we might repair or enhance the brain's function. Advances made possible by innovative techniques and technologies will improve treatment for people affected by neurological, degenerative and psychiatric disorders and deepen our understanding of how the brain creates consciousness and cognition.

The human brain consists of hundreds of billions of cells that form approximately one quadrillion connections and contains multiple, interacting levels of complexity. There are tens of thousands of different types of nerve cells, or neurons, each characterized by the unique pattern of genes they express, their shape and the connections they make with other cells.

Neurons possess two types of projections: one or more dendrites, which receive signals; and a single axon, which propagates processed signals to other cells. Through connections called synapses, neurons form complex, layered, overlapping networks of signals. The primary goal of neuroscience is to understand the formation and function of these neuronal circuits in order to determine how events at the cellular level are linked to higher-level cognitive processes such as language and memory—and how these processes break down in the many conditions that affect the brain.

The brain is responsible for generating thoughts, emotions and consciousness—everything that makes us human. The techniques available for direct study of the human brain have their limitations, however: functional magnetic resonance imaging (fMRI), for example, has low spatio-temporal resolution, and many of the experimental methods used in animals involve genetic engineering or are highly invasive, and are therefore not applicable to humans.

Understanding how activity within neuronal circuits gives rise to higher cognitive processes such as language, emotions and consciousness will thus require major technological breakthroughs. If this can be achieved, brain research will be revolutionized in the same way that recombinant DNA technology revolutionized biology in the early 1970s. Neuroscientists at the RIKEN Brain Science Institute (BSI) and elsewhere have made significant advances in recent years, making this an exciting and fast-moving research field.

The complexity of the brain and its wide variety of tasks and inaccessibility mean that neuroscientists need to combine many different approaches. At the BSI, this is achieved by bringing together teams from specialties including psychology, physiology, cell biology, genetics, mathematics, engineering and robotics. Research ranges from molecular analyses of synapses and neurons, and the use of multiple electrode arrays to record their activities, to large-scale neurogenetics studies and investigations of higher-order cognitive processes such as language and reasoning.

Through this interdisciplinary approach, BSI researchers have made significant discoveries, including major advances relating to brain development, structure and function, and the mechanisms that underlie neurodegenerative conditions such as Alzheimer's disease and psychiatric illnesses such as schizophrenia.

Growing knowledge and connections

Brain development in the fetus is complex and highly orchestrated. Billions of cells migrate through the developing tissue before extending their axons and dendrites to form extremely precise connections—a process that occurs largely after birth and is highly dependent upon sensory information.

Researchers at the BSI are making significant contributions to our understanding of brain development and recently determined the exact role of the only molecule known to be involved in orienting dendrites properly. Researchers are also using biochemical and molecular analyses to identify key signals involved in the formation of neuronal circuits. Combining these techniques with transgenic technology enables researchers to manipulate gene

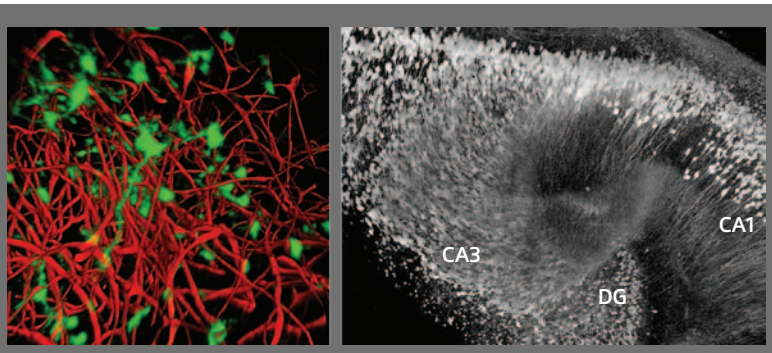

Figure 1: Seeing into the brain

The Scale reagent renders the brain transparent, allowing inspection of its fine structures down to an unprecedented level of detail. As a result, researchers can now quantify both the spatial organization of neural stem cells (green) and blood vessels (red) in the hippocampus—the brain's memory center—of the adult mouse (left) and reconstruct its substructures, including the dentate gyrus (DG), CA1 and CA3.

activity within specific cell types or circuits, while *in vivo* two-photon calcium imaging allows visualization of neuronal activity in living brain tissue. Thus, scientists can reveal the roles of these molecules in brain development.

Knowledge of the molecular mechanisms governing neurons—influencing axon guidance and growth—could eventually help researchers to better understand why nerve cells, and hence motor and sensory capabilities, fail to regrow after spinal cord injuries.

Shining light on memory

Memories are essential for our personal identity. Memory formation is thought to involve the strengthening of connections within a distributed network of neurons in the hippocampal region of the brain; memory retrieval is believed to occur by reactivation of the same networks.

A powerful new approach that can be used to study brain circuits is called optogenetics. This technique involves genetically engineering animals to express a light-sensitive protein that can directly control the electrical activity of specific populations of neurons. These target neurons can then be switched on or off—on a millisecond-by-millisecond timescale—using pulses of laser light delivered directly to the brain via fiber optic cables.

Researchers at the RIKEN–MIT Center for Neural Circuit Genetics are using optogenetics to investigate memory processes[1]. The researchers created a strain of mice that express this light-sensitive protein in the network of brain cells that become activated during memory formation. They were able to demonstrate that reactivation of these same cells stimulated retrieval of the memory.

By learning more about how memories are formed and retrieved, it may one day be possible to understand how these processes fail in patients with Alzheimer's disease and other conditions that involve memory loss.

Looking into the brain

The fine structure of the brain provides clues about its neural networks. To 'see' this structure, researchers typically label preserved samples of brain tissue with fluorescent markers and view them under the microscope. Since biological tissue scatters light—preventing light from penetrating any deeper than 1 millimeter—samples must be sectioned into thin slices. Slicing tissue in this way, then imaging and digitally reconstructing it, is expensive, laborious and only suitable for small volumes.

A better way to observe this structure is to render brain tissue completely transparent without altering the overall shape or proportions of the sample or losing the signals emitted by fluorescent markers. BSI researchers have achieved this by developing a chemical agent called Scale, which allows them to visualize labeled cells at unprecedented depth without the need for sectioning[2] (Fig. 1). This innovation will allow scientists to reconstruct entire neuronal networks at subcellular resolution, offering insight into the brain's circuitry.

Scientists are also able to study how these circuits function in living brains. BSI researchers are studying the mechanisms of higher brain functions, such as object recognition, decision-making and goal-directed behaviors, in non-human primates by blocking specific neuronal pathways, then using single-cell recordings to determine how each of the neural components contributes to the task.

Better understanding of the structure of the brain, its circuitry and its higher-level functions has numerous potential applications. For example, researchers worldwide are developing interfaces that allow paralyzed patients to control prostheses. Currently, most such devices are invasive, requiring implantation directly into the brain, and are unsuitable for a lifetime of use. BSI researchers are succeeding in making these techniques safer and more suitable for long-term application.

Brain research will not only contribute to furthering our knowledge of this complex organ but will also, through its application, allow doctors to more effectively treat the millions of people with neurological conditions and mental illness. The brain is the human body's center of learning and the organ that gives us our sense of identity. Studying its functioning will surely give us fresh insight into what it means to be human.

1. Liu, X., Ramirez, S., Pang, P. T., Puryear, C. B., Govindarajan, A., Deisseroth, K. & Tonegawa, S. Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature* **484,** 381–385 (2012).
2. Hama, H., Kurokawa, H., Kawano, H., Ando, R., Shimogori, T., Noda, H., Fukami, K., Sakaue-Sawano, A. & Miyawaki, A. Scale: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain. *Nature Neuroscience* **14,** 1481–1488 (2011).

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Physics

An exotic phase to manipulate spin

The experimental detection of an important quantum phenomenon in a semiconductor could lead to improved control of electron spin and the development of practical spintronics

In conducting materials, free electrons can move from one point to another, conveying their electrical charge to produce an electrical current. Electrons have another property, known as spin, that could be harnessed and manipulated in 'spintronic' circuits and has the potential to revolutionize the field of conventional electronics, leading to new functionalities and devices with enhanced performance.

Controlling electron spin states, however, is not as straightforward as controlling charge, making the development of practical spintronics a significant challenge. Quantum theory predicts that certain exotic energy states produced by the motion of electrons in solid matter could be used to control electron spin. Yet it is only recently that scientists have even been able to observe such exotic quantum energy states in the lab.

Hiroshi Murakawa and colleagues from the RIKEN Center for Emergent Matter Science, in collaboration with co-workers from the University of Tokyo and the SLAC National Accelerator Laboratory in the United States, have for the first time experimentally detected an elusive quantum property known as Berry's phase in a semiconductor[1].

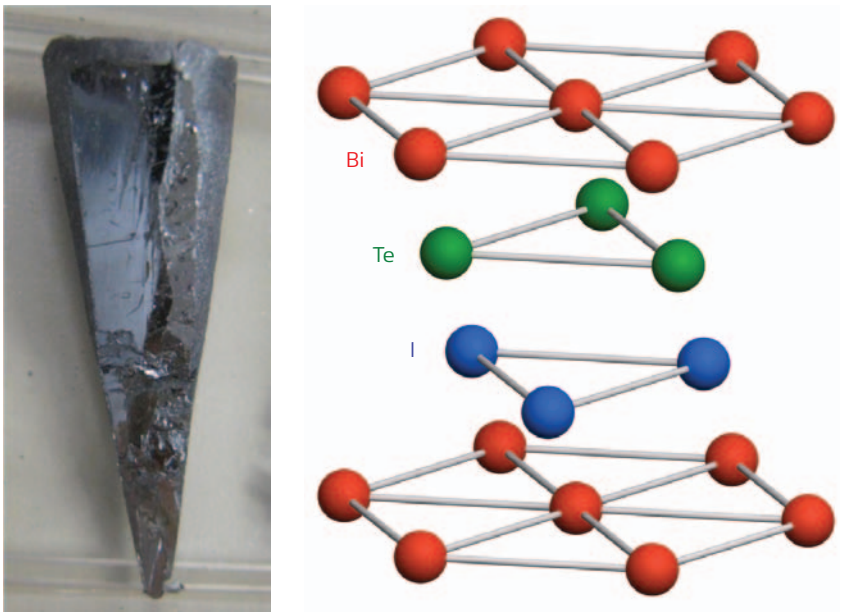

Figure 1: The crystal structure of the semiconductor bismuth tellurium iodide (BiTeI) (left) is made up of stacked layers of bismuth (Bi), tellurium (Te) and iodine (I) atoms (right).

Breaking waves to find the phase

"Berry's phase is a ubiquitous and fundamental notion in quantum physics," says Murakawa. Berry's phase gives rise to emergent phenomena such as the quantum version of the classical Hall effect—a transverse electrical current induced by an external magnetic field—as well as novel materials called topological insulators, which act as insulators in their bulk but conduct electricity on their surfaces.

"In quantum mechanics, an electron's motion, behavior and state are described by its wavefunction, which is characterized by an amplitude and a phase," explains Murakawa. "Typically, the phase reflects the time evolution of the electronic state and depends on energy and momentum terms. Berry's phase, on the other hand, is independent of time and reflects the system geometry. Geometrical information can therefore be encoded in the wavefunction and govern the motion of electrons."

It is these geometrical properties, such as the relative orientation of an electron's spin or momentum, that can give rise to Berry's phase. Most commonly, Berry's phase occurs in systems that are subjected to gradually changing cyclic processes that do not involve the transfer of heat or matter between the system and its surroundings. This condition is generally only met when the system has time to adapt its configuration to the changing conditions, such as when particles undergo rotation or translation.

"When the spin Berry's phase exists," says Murakawa, "novel phenomena such as spin-polarized charge flow without enegy dissipation can be realized. Despite

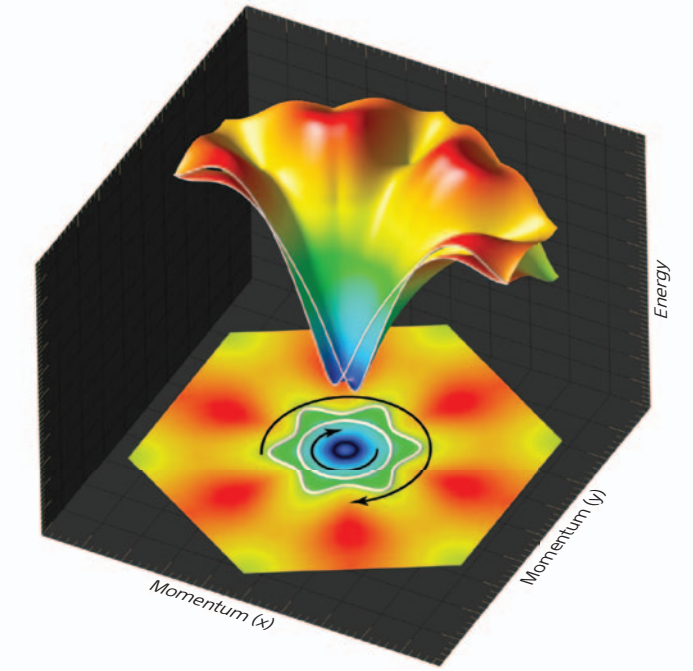

Figure 2: In BiTeI, electrons display strong Rashba spin–orbit coupling—the energy surface of the system is split into two sheets with opposite spin helicities (indicated by arrows).

Image courtesy of Mohammed Saeed Bahramy © 2014 Hiroshi Murakawa, RIKEN Center for Emergent Matter Science

its ubiquity and importance, however, experimental observation of Berry's phase stemming from electron spin is challenging."

Finding materials with the right split

An electron's spin can be either 'up' or 'down'. In most materials, electrons in spin-up states have almost identical energy to those in spin-down states. This leads to a phenomenon called energy degeneracy, in which the two states are difficult to distinguish. In their search for real manifestations of Berry's phase, Murakawa and his colleagues had to overcome the complication of energy degeneracy. They did so by focusing their attention on the semiconductor bismuth tellurium iodide (BiTeI)—a material with an unusual atomic structure consisting of stacked layers of its three individual component elements (Fig. 1).

The interfaces between the layers in BiTeI destroy the symmetry of the atomic lattice and give rise to a strong coupling between electron spin and motion, known as spin–orbit interaction. The overall result, known as Rashba splitting, is the removal of energy degeneracy, allowing different states to be easily observed (Fig. 2).

"Rashba semiconductors have not been applied practically until now, mainly due to the lack of an ideal system," says Murakawa. "The three-dimensional crystal structure of BiTeI allows a Rashba spin split to occur on a bulk scale, resulting in a huge number of spin-polarized electrons."

Observing oscillations

The researchers produced a series of single-crystal BiTeI samples with different electron carrier densities by growing the crystals at different temperatures. They then measured the electrical resistance of thin plates of the material. They also measured the Hall effect resistivity of the crystal under a strong magnetic field at temperatures of less than 2 kelvin—close to absolute zero.

The team was looking for the Shubnikov–de Haas (SdH) effect, a telltale sign of quantum behavior, by which free electrons behave like simple harmonic oscillators, fluctuating up and down in energy. This can be observed as an oscillation in magnetoresistivity with increasing magnetic field strength in materials at very low temperatures. In BiTeI, two sets of these oscillations corresponding to the two possible spin states could be clearly identified—the result sought after by Murakawa and his team. "The extremely large Rashba energy splitting in BeTeI enabled us to analyze each oscillation peak separately and disclose the spin Berry's phase clearly," he notes.

Murakawa suggests that it might be possible to induce persistent charge flow without energy loss by improving the crystal quality or optimizing the carrier concentration of BiTeI. The researchers are hopeful that they will eventually be able to control non-dissipative transport properties through the manipulation of Berry's phase.

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ABOUT THE RESEARCHER

Hiroshi Murakawa was born in Yamaguchi, Japan, in 1979. He graduated from the Department of Physics at Kyushu University in 2002 and obtained his PhD from Kyoto University in 2007. After spending four years working as a postdoctoral researcher on the Japan Science and Technology Agency's ERATO Multiferroics Project, in 2011 he joined the Correlated Electron Research Group at the RIKEN Advanced Science Institute (now part of the RIKEN Center for Emergent Matter Science). While at RIKEN, Murakawa's research focused on the transport properties of spin-polarized electronic systems. In October 2012, Murakawa moved to the Department of Physics at Osaka University, where he is an assistant professor.

Gut neurons get their marching orders

A new study identifies the signal that guides the migration and differentiation of enteric neuron precursors

Neural development involves the proliferation and migration of immature neurons, followed by their differentiation into the multiple cell types that make up the nervous system. These processes are poorly understood but are known to require the coordinated activity of dozens of transcription factors and signaling molecules. Hideki Enomoto, Toshihiro Uesaka and colleagues from the RIKEN Center for Developmental Biology have now identified a molecule that controls the migration and differentiation of a large population of neurons in the gut, known as the enteric nervous system (ENS)[1].

The ENS consists of two dense clusters of neurons: the myenteric ganglia (MG) and the submucosal ganglia (SMG) (Fig. 1). It is derived from the neural crest, a transient population of stem cells that arises along the top of the nervous system during early development. Although most of the peripheral nervous system forms relatively quickly, with cell migration followed immediately by differentiation, the ENS precursors that form the SMG are arrested in an immature state for a long period of time.

Glial cell line-derived neurotrophic factor (GDNF) is required for the proliferation and migration of premature ENS cells to form the MG. These cells are still responsive to GDNF at later stages of development but exactly why was unclear until now. Uesaka and his colleagues created a strain of genetically engineered mice in which the gene encoding the GDNF receptor can be inactivated at any time-point in development and made to express green fluorescent protein instead.

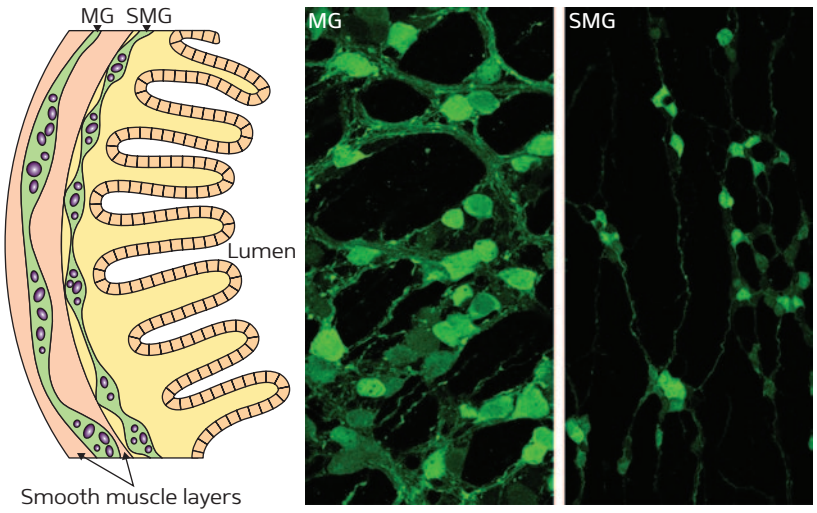

Figure 1: The majority of enteric nervous system (ENS) precursors form the myenteric ganglia (MG), while a subset of ENS precursors undergoes radial migration from the myenteric to the submucosal layer to form the submucosal ganglia (SMG).

The researchers deactivated expression of the receptor at various time-points during ENS development and traced the labeled cells under the microscope. The study revealed that GDNF signaling is essential for both primary MG and secondary SMG formation. They found that ENS precursors retained in the MG exhibited minimal activation of the GDNF downstream pathway. In addition, ENS precursors lacking the GDNF receptor maintained their undifferentiated state.

The findings suggest that low levels of GDNF signaling are crucial for maintenance of the undifferentiated state in ENS precursors, and that GDNF has a long-term impact on the driving force of ENS-precursor migration and neuronal differentiation. This was confirmed in experiments involving the reactivation of the GDNF receptor, which led the cells to resume their migration and neuronal differentiation.

"Hirschsprung's disease is associated with the genes for GDNF and its receptor, and neural crest stem cell functions regulated by GDNF probably contribute to its pathogenesis," says Uesaka. "Understanding the mechanisms regulating precursor-cell maintenance and neuronal differentiation could facilitate the design of cell-replacement strategies."

1. Uesaka, T., Nagashimada, M. & Enomoto, H. GDNF signaling levels control migration and neuronal differentiation of enteric ganglion precursors. *The Journal of Neuroscience* **33,** 16372–16382 (2013).

A model grass gets its genomic profile

A comprehensive cDNA library for a model grass species could help to improve crops for agriculture and biofuels

Figure 1: A new cDNA library for the purple false brome *Brachypodium distachyon* provides an invaluable resource for plant geneticists.

The grass species known as the purple false brome, *Brachypodium distachyon*, has great potential as a model plant for research due to its short generation time, small size, small genome and ease of breeding. These features make the grass species an attractive stand-in for less tractable but agriculturally important crops such as wheat and barley. However, to fully realize the potential of *Brachypodium* as a laboratory tool, scientists need more sophisticated genetic resources than are currently available.

Keiichi Mochida and colleagues from the RIKEN Center for Sustainable Resource Science have now constructed a comprehensive collection of all the DNA sequences in the *Brachypodium* genome that are transcribed into protein-coding messenger RNA (mRNA)[1]. This resource, known as a complementary DNA (cDNA) library, should help plant biologists to create more robust crops for food and biofuel production.

Model plant systems are essential for the genome-guided breeding of more resilient and higher-yielding crops. "*Brachypodium distachyon* is a model plant for analyzing the genetic functions and biological systems in temperate grasses, cool-season cereals and dedicated biofuel crops," explains Mochida. "The improved gene annotation based on full-length cDNAs provides essential information for identifying useful genes involved in various plant processes such as stress tolerance and biomass production," he says.

Mochida and his colleagues in the Biomass Research Platform Team and the Integrated Genome Informatics Research Unit extracted RNA from 21 different tissue samples of an inbred line of *B. distachyon* called Bd21. Using the 'CAP trapper' method developed more than a decade ago by scientists at RIKEN, Mochida's team obtained more than 78,000 short fragments of mRNA sequences. From this larger pool, the researchers assembled around 16,000 full-length 'clones' of cDNA.

With these clones in hand, the researchers updated and improved the existing *Brachypodium* genome sequence, adding structural information relating to some 10,000 genes across the plant's 5 chromosomes. Mochida's team found around 580 genes that had not been recognized as protein-coding stretches of DNA from previous bioinformatic analyses of the genome sequence and 362 novel genes identified for the first time in Bd21.

The researchers integrated this information with sequence data available from other economically important grass species in a public resource called the RIKEN Brachypodium FLcDNA database (RBFLDB). According to Mochida, the database now offers a "one-stop shop" for all genomic information in the Pooideae subfamily of grasses, which includes many major cereal crops. "*Brachypodium* now possesses all the features of a modern model organism," Mochida says.

1. Mochida, K., Uehara-Yamaguchi, Y., Takahashi, F., Yoshida, T., Sakurai, T. & Shinozaki, K. Large-scale collection and analysis of full-length cDNAs from *Brachypodium distachyon* and integration with Pooideae sequence resources. *PLoS ONE* **8,** e75265 (2013).

Finding meaning in gene expression 'noise'

Analysis of gene expression at the single-cell level reveals patterns and pathways that are impossible to detect in larger-scale experiments

Static models of gene regulation networks are inevitably oversimplified, indicating how one gene specifically switches certain target genes on while turning others off. However, the reality is much more dynamic and thus noisier. Jay Shin and colleagues from the RIKEN Center for Life Science Technologies have now monitored one such network in individual cells at different time-points during the complex process of cellular maturation[1].

The research team completed their study in a leukemia-derived cell line called THP-1 that can be chemically induced to differentiate into mature immune cells known as macrophages. Four years ago, scientists from the RIKEN-initiated FANTOM (Functional Annotation of the Mammalian Genome) consortium mapped out the genes that participate in this process, but the data were collected from large numbers of cells and thus sacrificed valuable information. "When cells are pooled," explains Shin, "the variation in gene expression is lost and only the mean expression of the population can be measured. By profiling single cells, we can quantify this natural variation."

Shin's team collected gene expression data from 120 individual cells at eight different time-points of THP-1 cell differentiation, focusing on a subset of 45 genes previously identified by the FANTOM research. Their analysis revealed how expression shifted for these genes over time (Fig. 1). However, the researchers also saw highly coordinated patterns of 'noise' in gene expression from individual cells at each time-point, revealing well-defined differentiation programs that can be initiated when the underlying gene networks are appropriately rewired.

Other patterns that would likely be lost in a bulk analysis also emerged. For example, subtle variations in the expression of 'master regulators' at the top of a gene network were found to trickle down to yield far greater noise in genes downstream. Noise analysis also revealed subsets of co-regulated genes, allowing Shin and his colleagues to identify gene subnetworks that either promote or constrain differentiation. These were connected via the *MYB* gene, which acts as a 'hinge' that can potentially trigger the activation of either subnetwork.

Shin hopes to extend the same kind of analysis to the entire cellular cohort of genes, but his team's success in characterizing expression dynamics in cancer cells also suggests clinical applications. "We wish to study this fascinating biological phenomenon in multiple cancer cell lines in response to various drugs now used in clinics," says Shin. "This will reveal the causal factors for variation in cellular response, which can be used to improve targeted treatment."

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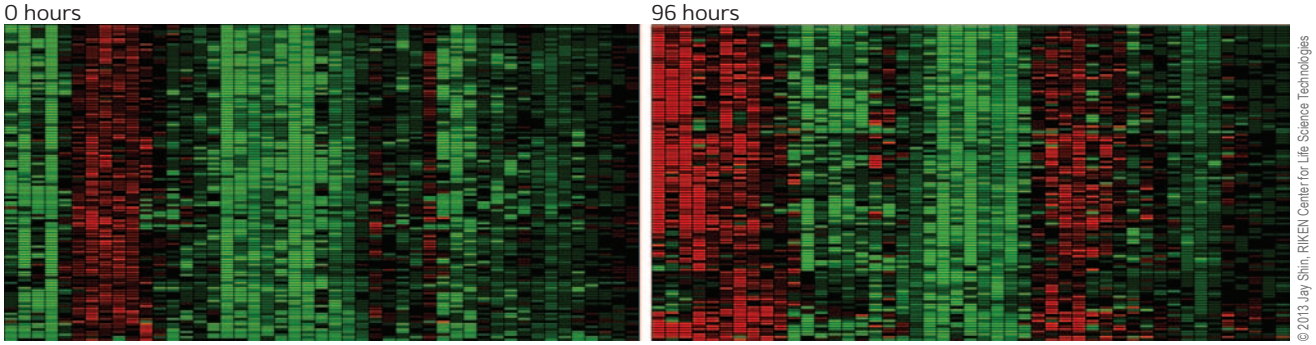

Figure 1: Analysis of changes in the expression of different genes (columns) in individual cells (rows) reveals clear inter-individual differences but also striking patterns associated with the differentiation process. Red and green squares denote higher and lower gene activity, respectively. These two maps were taken at 0 hours (left) and 96 hours (right), showing the different expression patterns at two cellular states.

Fighting asthma drug resistance

The poor efficacy of corticosteroid treatments in some patients with severe asthma could be overcome by blocking the action of an inflammatory protein

Current asthma treatments include drugs that open up the tubes of the lungs and corticosteroids that fight lung inflammation. Some patients, however, are stubbornly resistant to corticosteroids, limiting the therapies available to them. Shigeo Koyasu and Kazuyo Moro from the RIKEN Center for Integrative Medical Sciences, in collaboration with Hiroki Kabata of the Keio University School of Medicine and Koichiro Asano of Tokai University and co-workers, have now found that the inflammatory protein called thymic stromal lymphopoietin (TSLP) and its downstream signaling molecules play key roles in the resistance of natural helper (NH) immune cells, a member of group 2 innate lymoid cells (ILC2s), to the anti-inflammatory effects of corticosteroid treatment[1].

Asthma is a chronic disease of the airways characterized by persistent lung inflammation. The condition is known to be driven by the production of type 2 cytokines, such as interleukin (IL)-5 and IL-13, by NH cells.

The researchers developed a mouse model for this condition by administering an allergen and the pro-inflammatory cytokine IL-33 to mice. The treatment caused an increase in NH cells and mucus production in the lung, similar to the response observed in the lungs of asthmatic patients. Mice treated with corticosteroids did not show any improvement, indicating that the condition was resistant to the drug.

Koyasu and his colleagues found that the lungs of the mice that received the allergen and IL-33 also had higher levels of TSLP. Separately, they found that the addition of TSLP to NH cells cultured in the presence of IL-33 induced the proliferation of NH cells, even when corticosteroids were present. These findings suggest that TSLP causes NH cells to become resistant to the anti-inflammatory effects of corticosteroid treatment.

In other cell culture experiments the researchers found that in the absence of TSLP, NH cells died more readily under corticosteroid treatment, indicating that an antibody that blocks TSLP could eliminate corticosteroid resistance.

The team then discovered that in the presence of TSLP, NH cells activate the intracellular signaling protein STAT5. Pimozide, widely used as an antipsychotic drug, is known to inhibit STAT5. Experiments confirmed that pimozide did indeed restore the susceptibility of NH cells to corticosteroids in cell culture (Fig. 1).

The research provides strong evidence that pimozide could be used to eliminate resistance to corticosteroids for the treatment of asthma. "Because pimozide has already been in clinical use for a while, this drug could be applied to patients with corticosteroid-resistant severe asthma after appropriate clinical studies are conducted," explains Koyasu.

1. Kabata, H., Moro, K., Fukunaga, K., Suzuki, Y., Miyata, J., Masaki, K., Betsuyaku, T., Koyasu, S. & Asano, K. Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation. *Nature Communications* **4,** 2675 (2013).

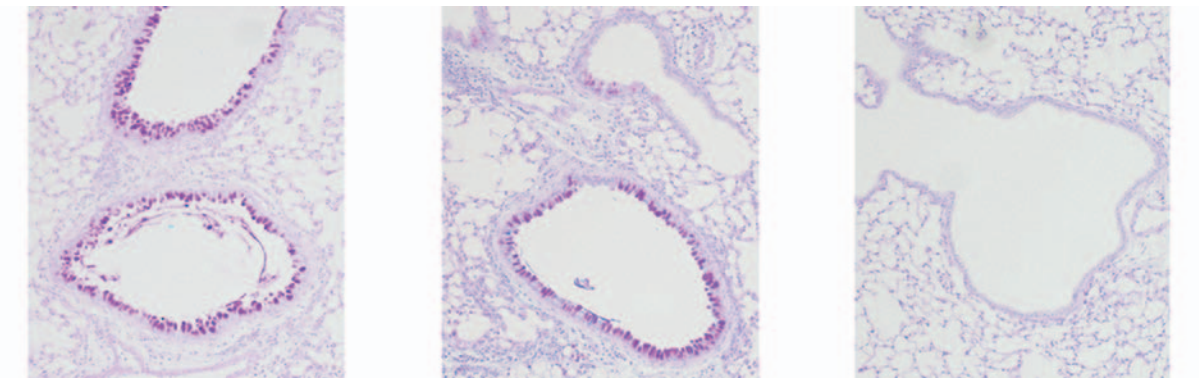

Figure 1: Asthmatic airway inflammation in mice induced by interleukin (IL)-33 and thymic stromal lymphopoietin (TSLP) protein (bright pink, left) cannot be reduced by steroid treatment (center) but can be reduced when steroid treatment is augmented by the STAT5 inhibitor pimozide (right).

A fleeting flash of light

A method for producing ultrashort pulses of intense light provides an important tool for studying the interaction between light and matter

Pulses of light are very useful for probing the inner workings of atoms, molecules and solids. Eiji Takahashi and co-workers from the RIKEN Center for Advanced Photonics, in collaboration with the Center for Free-Electron Laser Science in Germany, have now created a table-top light source that can generate attosecond optical pulses without the complicated level of stabilization required by alternative approaches[1].

The light source is produced by firing two high-power infrared laser pulses of about 30 femtoseconds—just billionths of a microsecond—in duration into a chamber filled with xenon gas. The mixed infrared light, called a two-color waveform, interacts with the xenon atoms, temporarily releasing electrons. When the electrons return to their host atoms, they produce an isolated extreme-ultraviolet (XUV) pulse through a process known as high-harmonic generation. The resultant pulses last just 500 attoseconds, some 60 times shorter than the input pulses. Furthermore, the central wavelength of the pulse is 40 nanometers and its peak energy is 1.3 millijoules—more than a hundred times greater than the energy achieved by previous approaches.

Takahashi's team demonstrated the utility of their light source by applying it to an analytical tool called pump–probe spectroscopy. In this powerful technique, a laser pulse—the pump—strikes an atom and excites its electrons. A second pulse then probes the state of the target at a later time. A full time-dependent picture of the atom and its electrons' reaction to the high-power laser light can then be constructed by varying the delay between the pump and probe pulses. The attosecond light pulses created by Takahashi and his team increase the temporal resolution of pump–probe spectroscopy to a level that enables researchers to gain a deeper understanding of ultrafast processes, such as ionization. "Our next step is to develop such an attosecond-pump/attosecond-probe experiment for observing and controlling electronic processes in atomic and molecular physics," says Takahashi.

The real strength of their novel light source, however, is the increase in peak pulse power. The strength of the interaction between light and matter usually scales linearly with the power of the laser light. The pulses of XUV light created by Takahashi and his team should open the door to investigating more complex effects in the nonlinear regime. "We also hope that it will be possible to extend our scheme to even shorter wavelengths, taking the light source into the soft x-ray region," says Takahashi.

Figure 1: Intense attosecond optical pulses provide a potent tool for studying how light interacts with solids, liquids and gases.

1. Takahashi, E. J., Lan, P., Mücke, O. D., Nabekawa, Y. & Midorikawa, K. Attosecond nonlinear optics using gigawatt-scale isolated attosecond pulses. *Nature Communications* **4,** 2691 (2013).

Tracing the protein assembly line

The cellular protein processing machinery is regulated by interactions between proteins that demarcate specific subsections of the cell's protein factory

Many newly synthesized proteins undergo a sequence of enzymatic modifications that enable them to do their jobs better. This process occurs within a series of membrane-bound structures called 'cisternae' that form the Golgi apparatus—an organelle that packages proteins before they are sent to their destination. Akihiko Nakano, Yasuyuki Suda and colleagues at the RIKEN Center for Advanced Photonics have now gained important insights into how protein movement through the Golgi apparatus is regulated[1].

When new proteins are produced, they are deposited into cisternae on the '*cis*' side of the Golgi. These proteins undergo a maturation process as they migrate toward the '*trans*' side of the Golgi before being released into the cell as fully processed proteins (Fig. 1). Nakano's team previously characterized this Golgi maturation process in yeast cells, resolving a long-standing debate in cell biology. "However, the molecular machinery of this cisternal maturation remained a mystery," says Suda.

Nakano, Suda and their colleagues are interested in enzymes known as Rab GTPases, which help to coordinate molecular trafficking within the cell. Different Rab GTPase proteins can serve as 'bar codes' that specifically identify cisternae in different zones of the Golgi. For example, the protein Ypt1 is found in the *cis*-Golgi in yeast cells, whereas Ypt32 resides in the *trans*-Golgi. "It has been speculated that the transition of Rab GTPases drives Golgi maturation because the transition from Ypt1 to Ypt32 coincides with this process," says Suda.

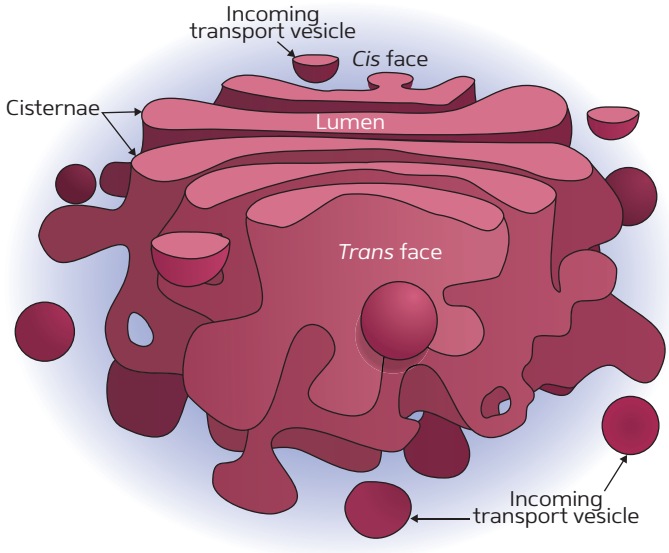

Figure 1: Protein processing in the Golgi apparatus is driven by a process of maturation, as stacked cisternae migrate in a *cis* to *trans* direction.

The researchers set out to determine the role of a third Rab GTPase, Ypt6. They found that Ypt6 generally resides in the middle region of the Golgi, and showed that although Ypt6 can be found alongside Ypt1, it is generally absent from Ypt32-bearing cisternae in the *trans*-Golgi. Rab GTPases are only bound to membranes when they are switched on. Subsequent experiments revealed that Ypt32 actively recruits another enzyme that turns Ypt6 off, thereby facilitating its removal from the membrane as cisternal maturation proceeds.

However, the absence of this Rab transition does not impair cell survival, indicating that this protein is not quite the last piece of this puzzle. "The resulting defects only made maturation a bit slower than usual, suggesting that other or perhaps multiple regulatory mechanisms drive Golgi maturation," says Suda. To delve more deeply into these processes, Nakano's lab has devised an innovative microscopy platform that allows the researchers to simultaneously observe multiple proteins in living cells in detail. "Using this device, we'd like to clarify the fundamental molecular machinery underlying protein trafficking."

1. Suda, Y., Kurokawa, K., Hirata, R. & Nakano, A. Rab GAP cascade regulates dynamics of Ypt6 in the Golgi traffic. *Proceedings of the National Academy of Sciences USA* **110,** 18976–18981 (2013).

Exposing the secret pathways behind photosynthesis

New insights into the behavior of photosynthetic proteins from atomic simulations could hasten the development of artificial light-gathering machines

The protein complex known as photosystem II splits water molecules to release oxygen using sunlight and relatively simple biological building blocks. Although water can also be split artificially using an electrical voltage and a precious metal catalyst, researchers continue to strive to mimic the efficient natural process. So far, however, these efforts have been hampered by an incomplete understanding of the water oxidation mechanism of photosystem II. Shinichiro Nakamura from the RIKEN Innovation Center and colleagues have now used simulations to reveal the hidden pathways of water molecules inside photosystem II[1].

At the heart of photosystem II is a cluster of manganese, calcium and oxygen atoms, known as the oxygen-evolving complex (OEC), that catalyzes the water-splitting reaction. Recent high-resolution x-ray crystallography studies have revealed the precise positions of the atoms in the OEC and of the protein residues that contact the site. While this information has yielded important structural clues into photosynthetic water oxidation, the movements of water, oxygen and protons within the protein complex are still the subject of much speculation.

To resolve this problem, researchers have turned to molecular dynamics (MD) simulation, a technique that models the time-dependent behavior of biomolecules using thermodynamics and the physics of motion. While previous MD simulations of photosystem II have involved the use of approximate models that focus only on protein monomers or the main OEC components, Nakamura's team took a different approach. "Our hypothesis was that we cannot understand the mechanism of oxygen evolution just by looking at the manganese-based reaction center," he says. "Therefore, we carried out a total MD simulation, without any truncation of the protein or simplification."

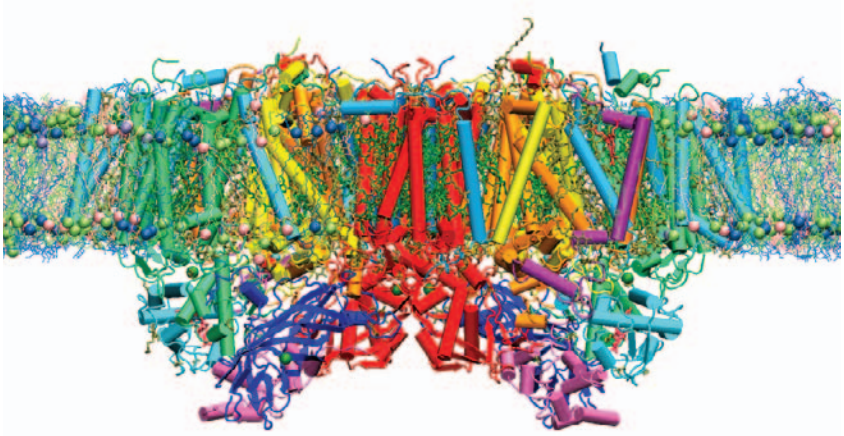

Figure 1: Computational simulations of photosystem II inside a realistic, water-containing membrane reveal the existence of previously hidden molecular pathways that play critical roles in photosynthesis.

In their simulation, the team embedded an exact model of photosystem II inside a thylakoid—a lipid and fatty-acid membrane-bound compartment found in the chloroplasts of plant cells. After initial computations confirmed the reliability of their model, the researchers performed a rigorous MD simulation of the protein–membrane system in the presence of more than 300,000 water molecules (Fig. 1). "The results indicated that water, oxygen and protons move through photosystem II not randomly but via distinct pathways that are not obviously visible," says Nakamura.

The pathways revealed by the simulations are delicately coupled to the dynamic motions of the photosystem II protein residues. While such intricate activity is currently impossible to reproduce artificially, the researchers suspect that combining quantum-chemical calculations with MD simulations could help to unlock the mysterious principles behind the highly efficient oxygen-evolution reactions of this remarkable biological factory.

1. Ogata, K., Yuki, T., Hatakeyama, M., Uchida, W. & Nakamura, S. All-atom molecular dynamics simulation of photosystem II embedded in thylakoid membrane. *Journal of the American Chemical Society* **135,** 15670–15673 (2013).

Quantum dots made to measure

A clever technique makes it possible to measure the intrinsic properties of quantum dot transistors

Transistors are one of the most important devices in electronics and lie at the heart of modern computing. The progressive miniaturization of transistors is rapidly approaching the atomic scale, where even the tiniest imperfection can have a significant effect on performance. Keiji Ono and colleagues from the RIKEN Low Temperature Physics Laboratory have now developed a method for measuring the operational characteristics of single-atom 'quantum dot' transistors without the influence of surrounding imperfections[1].

When a pure material is implanted with isolated atoms of another element, the impurity atom can behave like a quantum dot, with properties quite different to its host matrix. Quantum dots can form the basis of transistor operation—switching an output on or off, depending on the state of an input—and can facilitate the transport of electrons through the transistor even when electron transport through the surrounding material, usually silicon, is blocked. In this configuration, while all electrons pass through the quantum dot, they can do so only one at a time. This makes the quantum-physical properties of the quantum dots dominant in the transistor's operation, producing a characteristic diamond shape in the measured current–voltage relationship.

Single-electron transport through the transistor, however, is very sensitive to external perturbations. Impurities in other parts of the transistor can cause stray electrical fields that act as quantum dots and thus influence the electrical behavior of the transistor and the appearance of the diamond shape in the electrical curves.

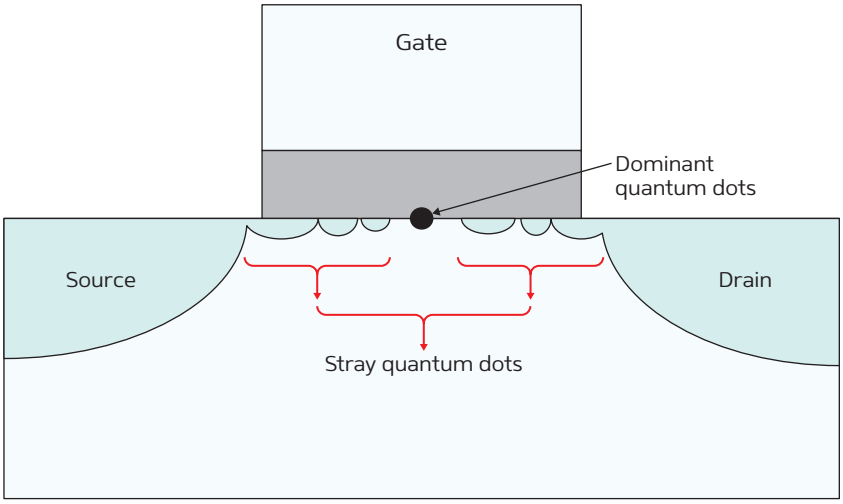

Figure 1: Structure of a transistor based on a quantum dot. One at a time, electrons flow from the source to the drain through the quantum dot, depending on the potential of the gate electrode. The properties of such a transistor are strongly affected by the presence of stray quantum dots in the transistor channel.

To counter such effects, Ono and his colleagues developed a measurement technique that allows them to quantify the effects of these 'stray' quantum dots so that the true properties of the main quantum dot can be isolated. The method is based on measurements of the transistor performance at various electrical voltages, which are analyzed using an electron transport model that incorporates the electrical effects of stray quantum dots. Among many uses, this information helps researchers to understand what voltages need to be applied to the transistors in order to optimize the single-electron transport regime.

Although the quantum properties of electron transport through quantum dot transistors only appear at low temperatures, understanding the processes involved is also important for the optimization of regular transistors at room temperature, which are known to be affected by the presence of single defects in the transistor channel, says Ono. "We know quite a lot about quantum dots. Applying quantum dot physics to commercial transistors is challenging but could have very useful implications."

1. Ono, K., Tanamoto, T. & Ohguro, T. Pseudo-symmetric bias and correct estimation of Coulomb/confinement energy for unintentional quantum dot in channel of metal-oxide-semiconductor field-effect transistor. *Applied Physics Letters* **103,** 183107 (2013).

Amplifying communication between neurons

The leak of potassium ions into the synaptic cleft between neurons can amplify synaptic signaling

Neurons send signals to each other across small junctions called synapses. Some of these signals involve the flow of potassium, calcium and sodium ions through channel proteins that are embedded within the membranes of neurons. However, it was unclear whether the flow of potassium ions into the synaptic cleft had a physiological purpose.

An international team of researchers including Alexey Semyanov from the RIKEN Brain Science Institute has now revealed that potassium ions that leak out of channel proteins and spill into the synapse augment synaptic signaling between neurons, potentially fulfilling a reinforcement mechanism in learning and memory[1].

Synaptic communication between neurons begins when calcium ions enter the axon terminal of one neuron—the presynaptic neuron—causing the release of neurotransmitter molecules, such as glutamate, which travel across the synaptic cleft and bind to receptor proteins on the surface of the receiving or postsynaptic neuron (Fig. 1). When the glutamate binds to a receptor known as the NMDA receptor, a channel in the receptor protein opens and calcium flows in, which initiates activation of the postsynaptic neuron.

Semyanov and his colleagues found that the opening of the NMDA receptor channel on the postsynaptic neuron also allows potassium ions to flow out of that neuron and into the synaptic cleft. Blocking the NMDA receptor prevented the rise in potassium ions within the synaptic cleft.

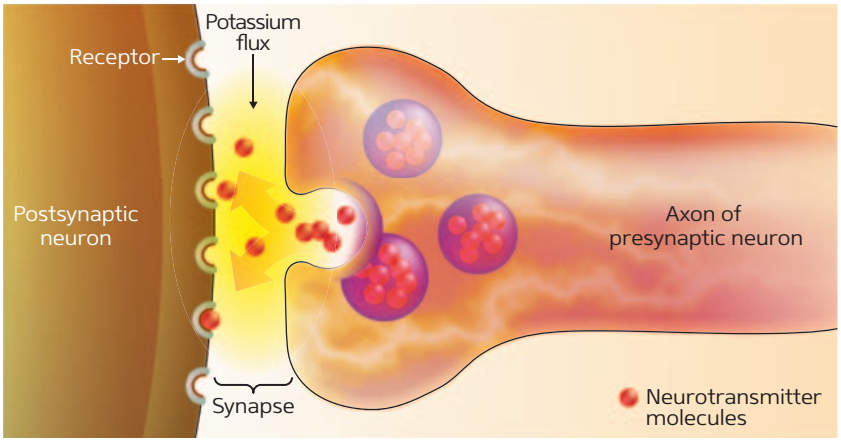

Figure 1: Synaptic signaling occurs when neurotransmitter molecules (glutamate) released by the presynaptic neuron travel through the synaptic cleft to activate glutamate receptors, including NMDA receptors, on the postsynaptic neuron.

Image courtesy of the US National Institute on Aging

The NMDA receptor is generally blocked by magnesium ions, but these ions can be released from the receptor channel upon repetitive stimulation of the postsynaptic neuron. Through mathematical modeling and subsequent experiments, Semyanov and his colleagues found that potassium levels in the synaptic cleft could increase dramatically on removal of magnesium or during repeated activation of the postsynaptic neuron.

The rise in potassium in the synaptic cleft was shown to increase calcium entry into the presynaptic neuron axon terminal when the postsynaptic neuron was stimulated, and enhanced the probability that the glutamate neurotransmitter would be released from the presynaptic neuron. In this way, repeated activation of a given neuronal network, such as during learning, could augment the strength of communication between neurons, making it more likely that a given stimulus would trigger the activation of postsynaptic neurons.

"New memories are associated with long-term changes in synaptic strength following repetitive activation of the synapse, commonly known as synaptic plasticity," explains Semyanov. "Potassium accumulation and the consequent increase in probability of glutamate release can potentially aid the induction of synaptic plasticity, thus facilitating learning and memory," he says.

1. Shih, P.-Y., Savtchenko, L. P., Kamasawa, N., Dembitskaya, Y., McHugh, T. J., Rusakov, D. A., Shigemoto, R. & Semyanov, A. Retrograde synaptic signaling mediated by K^+ efflux through postsynaptic NMDA receptors. *Cell Reports* **5,** 941–951 (2013).

Nuclear islands of deformation

The observation of unexpectedly deformed neutron-rich magnesium nuclei prompts a rethink of nuclear shell structure

Although much is known about atoms and their nuclei, scientists continue to make surprising discoveries as they probe the properties of some of the more exotic isotopes. Pieter Doornenbal from the RIKEN Nishina Center for Accelerator-Based Science (RNC) and co-workers have made another such discovery with the observation that magnesium nuclei with a large number of neutrons appear to lose the nuclear shell structure that has become fundamental to our understanding of the nucleus[1].

The protons and neutrons that make up an atomic nucleus are kept together by a balance of nuclear forces. When the number of neutrons is similar to the number of protons, the nucleus is generally stable and the nucleons arrange themselves in shells as a consequence of the laws of quantum mechanics.

Nuclear physicists now widely accept that nuclei with 2, 8, 20, 28, 50, 82 or 126 neutrons or protons are particularly stable due to the complete filling of these shells. Nuclei with such 'magic numbers' of protons or neutrons are spherical, whereas nuclei with numbers of nucleons that diverge from these magic values are increasingly deformed.

Doornenbal and his colleagues investigated the shape of magnesium nuclei with 22, 24 or 26 neutrons—a significant imbalance of neutrons against magnesium's 12 protons. "Studying such nuclei is now possible thanks to the RNC's Radioactive Isotope Beam Factory, which provides the world's highest-intensity radioactive isotope beams," says Doornenbal. The results indicate that the magic numbers for neutron-rich nuclei—and hence the filling of nuclear shells—might differ from those of the naturally occurring stable nuclei, in which the numbers of protons and neutrons are roughly equal.

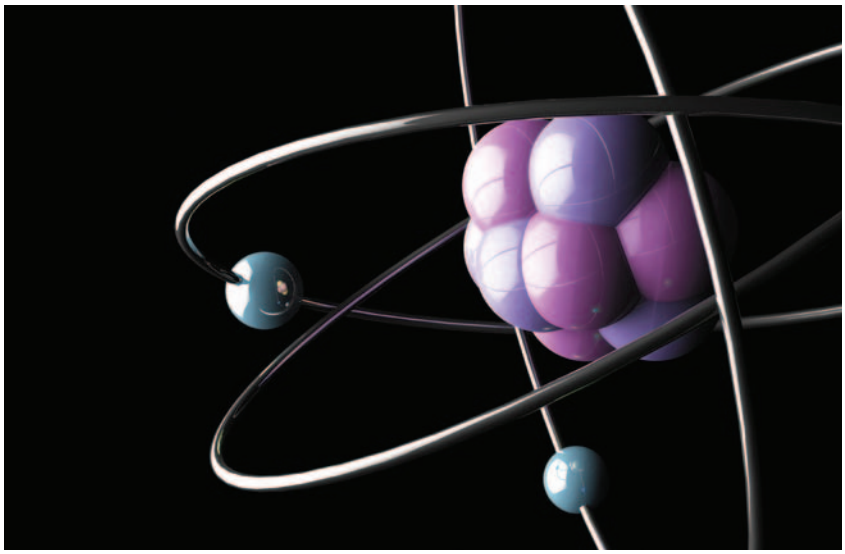

Figure 1: The shape of neutron-rich magnesium nuclei challenges the assumption that 'magic numbers' are the same for all nuclei.

The beams of magnesium nuclei were produced by first bombarding a high-energy beam of calcium nuclei against a thin beryllium target. The collision created a multitude of different nuclei that were then screened using magnetic fields to select precursor nuclei—aluminum-37, aluminum-39 and silicon-40. The desired magnesium nuclei were then obtained by bombarding the precursor nuclei against a carbon target to knock out additional nucleons.

The researchers probed the shape of the magnesium nuclei by measuring the high-energy electromagnetic waves that they emit. By comparing these results to theoretical calculations and previous experimental work, the team inferred a large 'island' of deformation in the isotope chart for neutron-rich nuclei with 20 to 28 neutrons. "This behavior is also expected to occur for larger magic numbers," says Doornenbal. "However, we do not yet have the experimental tools to study it in these regions."

1. Doornenbal, P., Scheit, H., Takeuchi, S., Aoi, N., Li, K., Matsushita, M., Steppenbeck, D., Wang, H., Baba, H., Crawford, H. *et al.* In-beam γ-ray spectroscopy of ^{34,36,38}Mg: Merging the N = 20 and N = 28 shell quenching. *Physical Review Letters* **111,** 212502 (2013).

Creating 'super trees'

TAKU DEMURA

Team Leader
Cellulose Production Research Team
RIKEN Center for Sustainable Resource Science

In plants, a network of water-conducting cells with thick cell walls located in the vascular tissue, known as xylem vessels, transports water from the roots. Taku Demura at the RIKEN Center for Sustainable Resource Science has identified the genes that control the differentiation of xylem vessels and other thick-walled wood cells in vascular tissue. Demura is creating 'super trees' that will contribute to the efficient use of biomass resources by using the identified genes to modify cell wall thickness and alter cell wall composition.

Structure of the plant cell

One of the features that distinguish plant cells from animal cells is the presence of a cell wall. While the cytoplasm of both plant and animal cells is enclosed by a cell membrane, plant cells also possess a cell wall, which surrounds the cell membrane and can be multilayered. Most plant cells possess a thin primary cell wall that serves to protect the cell and to maintain its shape. And some plant cells have an additional, very thick secondary cell wall—as exemplified by wood cells, the main target of Demura's research.

There are two types of wood cells: vessels, through which water is transported, and fibers, which support the plant body (Fig. 1). Vessels have hollows that, like tubes, are arranged in longitudinal rows, forming pathways for water. In 1980, Hiroo Fukuda—Demura's mentor at Tohoku University and currently a professor at the University of Tokyo—showed that the plant hormones auxin and cytokinin are required for the differentiation of vessels. "However, these plant hormones do not always function to induce the differentiation of vessels," explains Demura. "The detailed mechanism for controlling the differentiation of vessels remained unknown. Hence, we conducted research to find the genes that serve as the switches for the differentiation of vascular cells."

Turning on vascular cell differentiation

Demura established the Laboratory for Gene Regulation at the RIKEN Plant Science Center, now part of the RIKEN Center for Sustainable Resource Science (CSRS), in 2000. He conducted research to determine when and which genes work in the process of vessel differentiation using a cell culture system based on the flowering plant *Zinnia*, which he had been familiar with since he was a graduate student. "We uncovered some 100 genes that we believed to contain switches that control vessel differentiation. However, our experiments with *Zinnia* did not allow us to narrow down the range of these genes."

Therefore, Demura and his colleagues decided to study the experimental model plant *Arabidopsis thaliana*. In 2000, *Arabidopsis* became the first higher plant to have its genome sequence determined. Genomes contain the hereditary genetic information of a particular organism,

including the physical location of its genes within the genetic material. As a result, the researchers could easily find out which genes were present in the *Arabidopsis* genome as well as their location. In addition, the team was able to take advantage of established genetic transformation techniques for enhancing and suppressing gene expression in *Arabidopsis*.

Demura's team developed an experimental system in which cultured *Arabidopsis* cells could differentiate into vessels and conducted extensive investigations to identify the genes that were influencing the differentiation. They discovered that some 200 genes were involved in the differentiation process, including genes with similar sequences to those that had been found in the cultured *Zinnia* cells—hinting at similarities in terms of their function. In 2005, extensive analysis by the team revealed that forced expression of the genes encoding the plant-specific transcription factors VND6 and VND7 induced vessel differentiation in cells that did not otherwise differentiate into vessels. They also found that vessels do not form when VND6 and VND7 are functionally suppressed. "After many painstaking steps, we were able to find the genes that serve as switches for vessel differentiation," says Demura. "In addition, it turned out that the two genes controlled the process of producing the two types of vessels in distinct ways."

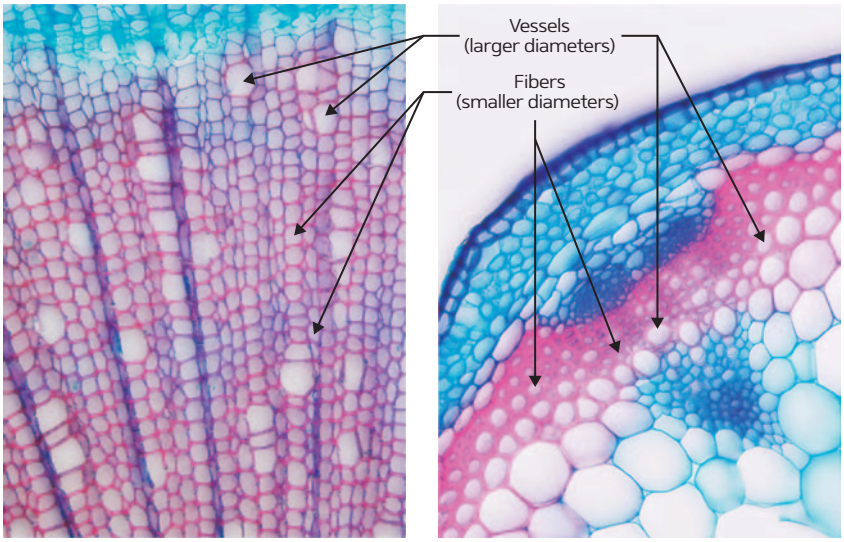

Figure 1: Cross-sectional views of the stem of a young poplar plant (left) and *Arabidopsis thaliana* (right). Wood cells with secondary cell walls appear in red.

Producing two types of vascular cells

There are two types of vessels: protoxylem vessels, whose cell walls contain spiral thickenings, and metaxylem vessels, whose cell walls are thickened in a pitted or reticulate pattern (Fig. 2). Initially, a plant forms protoxylem vessels then subsequently forms metaxylem vessels. But if metaxylem vessels form first, the plant is unable to grow normally. This is due to differences in the characteristics of the two types of vascular cells.

To produce the channels through which water can be transported, vessels become hollow, forming a thickened cell wall and ultimately dying upon reaching maturity. When cells surrounding the vessels expand, however, the dead vessels are unable to similarly increase their size, thus interfering with the growth of the plant. To prevent this, the plant initially produces the spiral-patterned protoxylem vessels, which are dead but still able to elongate, meaning that they can keep pace with the growth of the cells around them. After the surrounding cells cease their growth, the vessels no longer need to elongate. The plant then produces the more rigid, pitted or reticulated metaxylem vessels. "The difference between protoxylem and metaxylem vessels can be easily understood by comparing them to the elastic portion of a bendable straw and a hose, respectively," notes Demura.

The production of these two types of vessels must be controlled in distinct ways in order for normal plant growth to occur. "We showed that when the transcription factor VND7 is overexpressed, undifferentiated cells differentiate into protoxylem vessels, but when VND6 is overexpressed, these cells differentiate into metaxylem vessels."

In 2006, Demura and his colleagues demonstrated that the gene encoding the SND1 transcription factor, which is only expressed in fibers—the other type of wood cell—is important for the differentiation of fibers. The protein structure of SND1 is very similar to that of VND6 and VND7. Interestingly, by forced expression of SND1 in cells that originally lacked a thick secondary cell wall, a secondary cell wall can be formed. Conversely, when the function of SND1 is suppressed, the cell walls of fibers become so thin that the plant is no longer able to stand upright.

Four years later, Demura and his colleagues went on to discover VNI2, a protein that suppresses the function of VND7. Overexpression of VNI2 inhibits protoxylem vessel formation, resulting in broken vascular vessels.

"Elucidating the control mechanism for vessel differentiation was an important achievement in plant science," says Demura. "However, this is not our ultimate goal. I hope that this knowledge will find useful applications for society in general, rather than merely in the academic sector."

Super trees with improved biomass

Demura and his team first began to use the term *super tree* to introduce their work over ten years ago. "At RIKEN, a wide variety of useful genes have been discovered, including those involved in providing resistance to various stresses, such as drought and pests, and those involved in growth control," says Demura. "Trees can be transformed

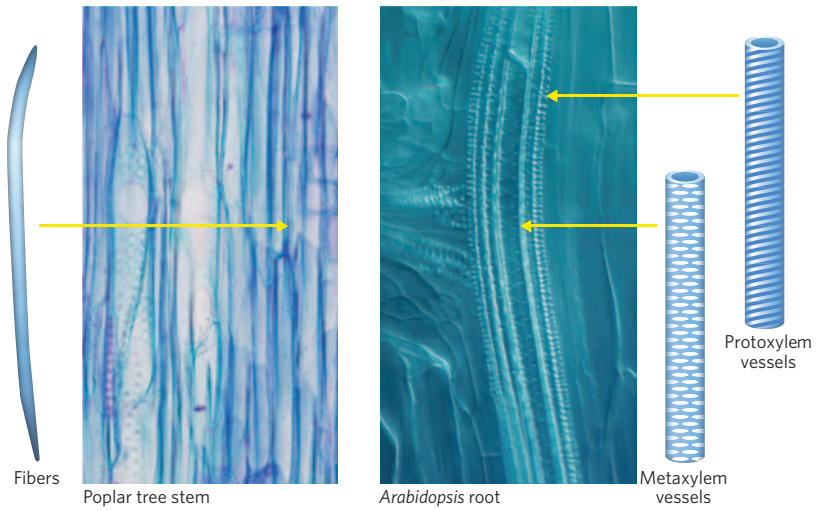

Figure 2: Types of wood cells

Wood cells contain two types of cells: fibers and vessels. Vessels include both protoxylem vessels, which have secondary cell walls thickened in spiral patterns, and metaxylem vessels, which are characterized by secondary cell walls that possess pitted or reticulate patterns. The panels show longitudinal sectional views of the stem of a poplar (left) and an *Arabidopsis* root (right).

with these genes to enhance their useful functions. We named these modified trees 'super trees'."

Traditionally, petroleum and other fossil resources have been used as energy sources and materials for manufacturing. However, the availability of fossil resources is limited and their incineration increases atmospheric carbon dioxide (CO_2), which contributes to global warming.

Plants absorb CO_2 and utilize solar energy to produce a wide variety of substances generically known as biomass. When CO_2 and solar energy are available, biomass essentially becomes renewable. In addition, the CO_2 emitted upon the incineration of biomass was previously a component of the atmosphere, so the total amount of CO_2 remains unchanged. Hence, to realize sustainability, society must make a modal shift from its dependence on fossil resources to the most efficient use of biomass.

"The most abundant biomass on land is trees, and tree biomass is mostly composed of the cell walls of wood cells," explains Demura. "I am confident that super trees created with the genes that control wood cell differentiation will help to resolve these problems."

The primary component of the plant cell wall is cellulose, accounting for 50 per cent of the total composition. Hemicellulose accounts for a further 25 per cent and lignin for the remaining 25 per cent. The structure of the cell wall can be compared to that of reinforced concrete, with cellulose equivalent to the reinforcing steel bars, hemicellulose the wires that join the reinforcing steel bars, and lignin the concrete that fills the gaps.

Cellulose and hemicellulose are polysaccharides consisting of a chain of sugar units. They can be degraded, or saccharified, to sugars with a broad range of uses, including bioethanol and raw materials for plastics. Lignin, on the other hand, is barely degradable and the maximum amount possible must be removed in order to allow cellulose to be extracted from the cell wall. The foodstuffs sugarcane (containing sucrose) and corn (containing starch) are currently also used as raw materials for bioethanol. However, if successfully saccharified, cellulose derived from sugarcane bagasse—a fibrous material that remains after sugarcane is crushed—and corn stalks, rice straw and wood could serve as bioethanol and raw materials for plastics that do not compete for resources that could otherwise be used for growing food.

"We aim to freely control the formation of cell walls by enhancing or suppressing the functions of the genes that control wood cell differentiation to increase the cell wall thickness, change the cellulose–lignin ratio and allow cellulose to be more easily digested into sugars," says Demura.

From *Arabidopsis* to poplars

To link the findings in *Arabidopsis* to the creation of super trees, Demura is conducting research using the poplar tree. In 2006, the genome of the poplar was fully determined, and genetic transformation of the tree was well established.

Demura has already started an experimental study to transfer the genes for wood cell differentiation that he discovered in *Arabidopsis* to poplars. When VND6 is introduced, some of the epidermal cells of poplar leaves differentiate into metaxylem vessels, and the introduction of VND7 causes some leaf cells to differentiate into protoxylem vessels—the same as occurs in *Arabidopsis* (Fig. 3). However, the team also found an unexpected result. "When VND7 was transferred to poplars, patterns similar to those of vessels appeared on the cell walls of the fibers—a change that allowed cellulose

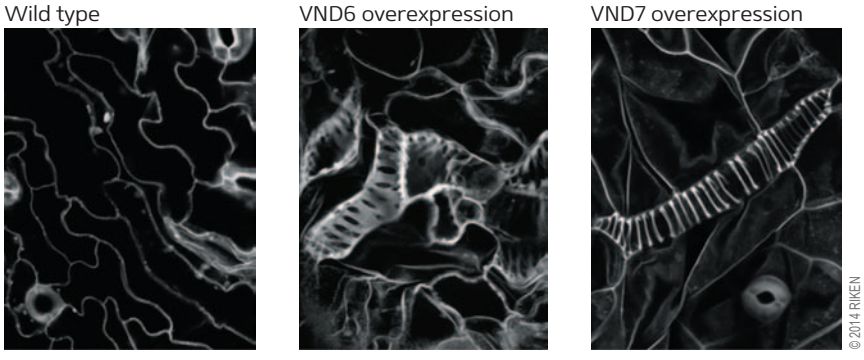

Figure 3: Induction of vessels in poplar leaves due to overexpression of VND6 and VND7

When VND6 is overexpressed in poplar leaves, some epidermal cells differentiate into pitted or reticulated metaxylem vessels (center). When VND7 is overexpressed, the cells differentiate into spiral-patterned protoxylem vessels (right).

Figure 4: Genetically modified poplar trees and the open-field experimental station at Nanjing Forestry University

Collaborators at the Nanjing Forestry University in China are working to transfer genes for wood cell differentiation sent by the RIKEN Cellulose Production Research Team into poplar trees. Test cultivation will take place in the university's open-field experimental station (bottom).

to be more readily used," says Demura. Saccharification enzymes are not thought to readily enter the cell wall of fibers due to the smooth surfaces of such cells. It is possible that surface undulations could allow the saccharification enzymes to enter the cell wall of fibers with greater ease, facilitating cellulose saccharification. However, as the introduction of VND7 also leads to thinning of the secondary cell wall, a balance between increasing the ease of access for enzymes while minimizing thinning must be achieved.

Outdoor cultivation in China

Demura and his Cellulose Production Research Team at the CSRS are growing genetically modified poplars in a greenhouse at RIKEN. At most, these trees grow to about two meters tall. Unfortunately, this means that the effect of the introduced genes cannot be fully understood and evaluated without growing the trees in an open-field experimental station.

In 2005, Demura began to search for a research partner who could assist his team in growing the super trees outdoors. After collecting a vast amount of information on research into genetic recombination for poplars from all over the world, he decided to reach out to researchers in China, where genetically modified poplars with pest and cold resistance have already been planted. Coincidently, RIKEN was advocating the Asia strategy of its Biomass Engineering Program to accelerate research cooperation with Asian countries—something that boosted the joint project. After visiting a number of universities and research institutes, Demura chose to collaborate with Nanjing Forestry University.

Currently, Demura is preparing to grow poplars transformed with the stress resistance genes discovered by Kazuo Shinozaki, director of the CSRS, and his colleagues in the open-field experimental station in China. "We have already sent the genes for wood cell differentiation to Nanjing Forestry University and they are working to transfer the genes to poplars. Following testing of poplars transformed with the stress resistance genes, poplars transformed with our genes for wood cell differentiation will also be tested at Nanjing," (Fig. 4).

Moving between basic and applied research

According to Demura, while steady progress has been achieved in the creation of super trees, a major problem still remains. "We are unable to freely control cell wall formation as our present understanding of the mechanism for controlling wood cell differentiation is insufficient."

Researchers now believe that more than one 'sub-switch' is present downstream of the main switches for wood cell differentiation that Demura discovered. In addition, there are likely to be switches that activate the main switches. While working to apply the wood cell differentiation genes to poplars, Demura is concurrently conducting research in *Arabidopsis* to precisely determine how the expression of VND6, VND7 and SND1 is controlled. "I assume that the switches do not work in the on–off mode, but in the dial mode," he explains. "To achieve super trees, fine adjustments are essential to obtain a delicate balance by combining the intensities of multiple switches."

Demura began his work to develop super trees in 2003. "I was invited to give a lecture at an international academic meeting on tree biotechnology. Through this opportunity, I realized for the first time that our research achievements in *Zinnia* and *Arabidopsis* were applicable to woody plants and of potential benefit to society."

Demura notes that the study of trees requires both a wide space and a long time. "Our strategy is to narrow down the useful genes using *Arabidopsis*—a common plant with a short lifecycle that can be grown in laboratory settings—and to apply the selected genes to trees," he says. "Findings in the trees can be verified after feedback to *Arabidopsis* to obtain data that should help to improve the trees." This ability to move between basic research in the lab and applied research in the field is proving to be one of Demura's team's greatest strengths. Consequently, and despite the many other competing research groups around the world with interests in tree genetic recombination, Demura's *Arabidopis*-based strategy looks set to help him continue to obtain world-leading results.

ABOUT THE RESEARCHER

Taku Demura graduated from Tohoku University in 1990 and obtained his PhD in 1995 from the same institution. From 1995 to 2000 he served as an assistant professor at Tohoku University and the University of Tokyo. In 2000, he joined the RIKEN Plant Science Center as a laboratory head. In 2010, he was appointed as team leader of the Cellulose Production Research Team at the RIKEN Center for Sustainable Resource Science. His research focuses on the regulation of woody cell differentiation, which extends to the applied biotechnology of trees, to improve the utility of woody biomass to provide solutions to environmental problems and other global issues.

In the business of investigating antimatter

STEFAN ULMER

Initiative Research Scientist
Ulmer Initiative Research Unit

Please describe your role at RIKEN.

My research unit is leading the Baryon Antibaryon Symmetry Experiment (BASE) at CERN (European Organization for Nuclear Research), which aims to measure the magnetic moments of the proton and antiproton with a fractional precision of one part per billion or greater. The experiment will be conducted at CERN's antiproton decelerator in Switzerland and is strongly supported by RIKEN. My unit is also involved in the antihydrogen research driven by Yasunori Yamazaki's Atomic Physics Laboratory at RIKEN.

How did you become interested in your current field of research?

High-resolution observations of antimatter offer very sensitive tests for CPT (charge, parity and time reversal) invariance, which is the most fundamental symmetry in the standard model of particle physics. Achieving such high-precision observations requires the construction of very accurate apparatus and the development of novel, pioneering techniques. I am fascinated by both the pioneering technical work required to develop ultraprecise machines as well as the strong impact of our tests upon the field of physics. The research is very important and exciting.

What made you decide to become a scientist?

I chose physics because it is both challenging and useful. In principle, with a good physics background you can work in all of the technical, engineering and mathematical fields. Physics offers insight into the symmetries of nature and engages in interesting philosophical questions.

How and when did you join RIKEN?

I was keen on getting into the antimatter business because antihydrogen physics is an exciting area of research and I had already developed techniques to measure the magnetic moment of the proton, which can also be applied to the antiproton. I was impressed by Yamazaki's personality and knowledge and became convinced that working with him would be good for me. In 2011, I joined his lab at RIKEN as a foreign postdoctoral researcher, and in April 2012 I received an Initiative Research Unit (IRU) grant.

What is the best thing about working at RIKEN?

The support available to researchers from all levels of staff at RIKEN—including IRU assistants, the Global Relations and Research Coordination Office, other principle investigators and the directors of centers—is impressive. Whenever I face difficulties there is immediate and strong support from many individuals.

Please tell us about your professional and personal goals.

My professional goal is to measure the magnetic moments of the proton and the antiproton with ultrahigh precision, and to develop a machine to refine the sensitivity down to parts per trillion and beyond. I also aim to apply sympathetic laser cooling of antiprotons to accelerate experiment cycles and improve antihydrogen production for high-precision measurement of ground-state hyperfine splitting of the antiatom. My personal goal is to enjoy life with my wonderful family.

How do you balance family life with your work at RIKEN?

Work–life balance is not an issue if work is fun. My family, specifically my wife Eva, is very supportive and generous.

CONTACT INFORMATION

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RIKEN Center for Life Science Technologies celebrates anniversary with symposium

On 17 February 2014, the RIKEN Center for Life Science Technologies (CLST) organized a symposium in Tokyo to commemorate the first anniversary of its founding. The CLST was established in April 2013 with the aim of integrating structural biology, genomic technologies and imaging science to create an advanced technological platform for research and development across the life sciences.

The symposium, titled "The New Life Science Universe—Towards Creating the Technological Base for Discovering Molecular Network Regulation," centered on new technology-based solutions that could help to deliver innovative and efficient treatments for the major diseases plaguing modern society, notably cancer and Alzheimer's disease, as well as lifestyle-related conditions, such as obesity and diabetes.

Yasuyoshi Watanabe, director of the CLST, welcomed the diverse gathering of more than 350 academics and industrial leaders and emphasized the need to promote stronger cooperation between academia and industry from an early stage. "The idea of human life science is at the core of our activities," he said. "Our aim is to serve society by developing technologies that will help medical innovation. This conference is an important step in connecting researchers, clinical doctors and companies to drive drug and diagnostic development."

Yasuyoshi Watanabe, director of the RIKEN Center for Life Science Technologies (CLST), called for stronger cooperation between research, academia and industry at a symposium organized to commemorate the establishment of the CLST.

Panelists at the symposium shared advances in cancer drug research and drug delivery systems that may ultimately enable doctors to apply targeted medication to diseased tissues without harming the surrounding healthy tissue. ■

RIKEN represents Japan at the 2014 AAAS Annual Meeting

RIKEN was one of a select group of research institutes and universities represented at the Japan Pavilion of the 2014 American Association for the Advancement of Science (AAAS) Annual Meeting, held in the United States on 13–17 February. The RIKEN exhibit offered keen individuals the latest information on research activities, recent partnerships with other research institutes and working at RIKEN.

Over 5000 members of the public—from children to high school students, teachers, young researchers, entrepreneurs and journalists—visited the Japan exhibit and interacted with the delegates present.

RIKEN and the World Premier International Research Center Initiative (WPI) program also participated in a workshop on 14 February entitled "Build a Career in Japan!" and coordinated by the Japan Science and Technology Agency. At the session, RIKEN presented employment opportunities and shared case studies, which offered insight into life as a researcher at RIKEN and highlighted the extensive support systems provided by RIKEN to foreign researchers. ■

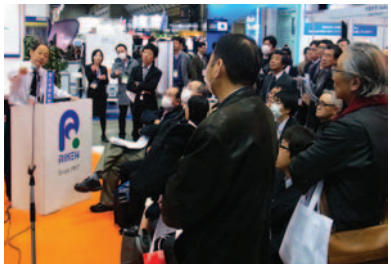
RIKEN exhibited at the Japan Pavilion of the 2014 AAAS Annual Meeting in Chicago in February.

RIKEN attends nano tech 2014

RIKEN hosted a booth at nano tech 2014 —the 13th International Nanotechnology Exhibition & Conference—held in Tokyo on 29–31 January. More than 45,000 individuals, including technological innovators and potential users, were drawn to the convention and immersed in a space that had been specially designed to encourage collaboration and industrial evolution.

RIKEN introduced its latest breakthroughs and technologies at the exhibition through 14 presentations on new materials, such as organic semiconductors with applications in solar cell technology, and devices, such as novel microscopes. The talks provided an opportunity to initiate partnerships with the private sector for potential commercialization.

Among the presentations, Shinichiro Nakamura from the RIKEN Innovation Center discussed photosynthesis and 'molecular music'—a method of presenting the vibrations of molecular bonds in the form of audible sounds. In addition, a representative from The NEXSYS Corporation, set up under the RIKEN Venture System, shared the company's experiences of commercializing surface grinding technology developed at RIKEN. ■



Shinichiro Nakamura from the RIKEN Innovation Center gave a well-attended presentation on photosynthesis and 'molecular music' at nanotech 2014 in Tokyo.

www.riken.jp/en/research/rikenresearch

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